REMARKS

Claims 31-39 and 41-61 are pending in the application; claims 31, 41, and 42 are rejected; claims 32-39 and 43-61 are withdrawn.

New claim 62 has been added that relates to collection of cells as a tissue sample; the feature is disclosed on page 13, 1st full paragraph.

Claim Rejections - 35 U.S.C. 112

Claims 31, 41 and 42 stand rejected under 35 U.S.C. 112, 1st paragraph, as failing to comply with the enablement requirement.

Examiner argues that the step of determining the receptivity as follows:

1. no ß7-hCG, ß6-hCG, and ß6e-hCG is detected: the endometrium is not receptive;
2. at least one of ß7-hCG, ß6-hCG, and ß6e-hCG is detected: the endometrium is receptive for implantation;

is not enabled by the specification.

The specification sets forth on page 11, last paragraph, that (emphasis added):

"The invention is based on the scientific finding that the level of expression of the genes of the type I-ß-hCG (ß7, ß6, eß6) in the normal secretory epithelium of the uterus lining (endometrium) or in the mononuclear cells of the peripheral blood represents a reliable indicator for a possible successful implantation. The higher the expression the better the chances for a successful implantation of a fertilized egg or an embryo."

The specification further sets forth on page 12, 3rd full paragraph, that (emphasis added):

"It has been **recognized** that the contents of ß6 hCG and ß7 hCG in the body's own epithelial tissue or blood cells determines the success of an implantation fundamentally and that therefore the knowledge of the amount of hCG ß7 and ß6, considered absolute or relative in knowledge of the quotient of hCG ß7, ß6 as numerator and hCG ß5, ß8, ß3 as denominator provides information in regard to the promising moment of implantation."

The specification further sets forth in the paragraph bridging pages 12 and 13 that (emphasis added):

"For diagnosing the receptivity of the endometrium, preferably tissue from the endometrium or from the cervical lining or peripheral blood is removed from the female patient and the analysis of the mRNA expression is determined in this blood or tissue sample with the method according to the invention. Based on the level of the determined mRNA expression of **ß7-hCG and/or ß6-hCG and/or eß6-hCG** it is then possible to draw conclusions in regard to the receptivity of the uterine for an embryo in the current or the subsequent cycle."

On page 13 (3rd full paragraph) it is stated, after two paragraphs that explain the procedures used, that

"A positive detection of mRNA of ß6-hCG, ß7-hCG or eß6hCG indicates that the endometrium differentiates in the direction toward implantation readiness."

Thus, the inventors have found that type I-ß-hCG (ß7, ß6, eß6) is an indicator for successful implantation and have set forth positively in the specification that mRNA expression of ß7-hCG and/or ß6-hCG and/or eß6-hCG is an indicator for implantation readiness.

It is also set forth in the paragraph bridging pages 13 and 14 that the same test as described previously (i.e., in relation to tissue taken from the cervix or the uterus) can be done with menstrual blood where a sufficient number of cells from the endometrium are present.

The inventors' findings are new and therefore cannot be found in literature predating the current invention. The one reference (*Coutifaris et al.*) cited by examiner that post-dates the current invention has nothing to do with hCG - it relates to histological dating of endometrial biopsy tissue (histological slides are evaluated by a pathologist; see page 1266, right column, last paragraph). This reference post-dating the instant invention does not relate to hCG determination and addresses an entirely different problem; therefore, any reference to hCG and its effects has no place in such a publication and the general remarks that more work should be done on molecular markers (hCG is not mentioned) cannot be construed as evidence that the method presented in this application is unpredictable in view of the prior art.

On page 16 of the specification, it is stated that:

"It can be assumed that the presence of hCG ß7, ß6, and ß6e is an indicator for an

optimal implantation. The lack of hCG ß7, ß6, and ß6e indicates the opposite: the possibility of implantation in this cycle is not to be expected."

Examiner states on page 6, first paragraph, of the office action that "At the very outset specification states "It can be assumed ...", reciting the text portion of page 16, lines 2-4. The examiner then proceeds to state that the specification only provides an assumption which forms the basis of the claimed method and that this assumption is not substantiated by any clinical data.

Applicant respectfully disagrees: page 16 is not the **outset** of the specification or disclosure. As discussed above, the specification sets forth several times that the invention is "based on the scientific finding ..." (page 11, 3rd to last line; page 12, lines 4/5) and that "It has been recognized that the contents of ß6 hCG and ß7 hCG...". The inventors have recognized and scientifically determined that ß7-hCG and/or ß6-hCG and/or eß6-hCG indicate by their presence implantation readiness while their absence indicates that implantation is not possible. The one time use of the word "assumed" cannot negate these clear and unequivocal statements made by the inventors.

Applicants respectfully submit that the present invention is based on the recognition that the expression of hCG (ß6, ß7, eß6) by the endometrium (the maternal tissue) is required in order for the embryo to implant in the uterus. The endometrial expression of hCG has a regulatory effect on the immune system. The endometrial expression of hCG prevents that the embryo (expressing also paternal proteins) is recognized as foreign by the maternal immune system and is rejected. That hCG has a regulatory effect on the immune system is supported by a several publications. hCG recruits regulatory T lymphocytes (in press) and induces a regulatory phenotype in dendritic cells (Wan, H. et al.; J. Leukoc. Biol. 2008 (April), pp. 894-901; Abstract attached). This means that detection of endometrial expression of ß6 or ß7 or eß6 hCG in a woman that is not pregnant correlates with the receptivity of the endometrium for an embryo, i.e., a natural or artificial fertilization has excellent chances of success.

In healthy endometrium expression of hCG (ß6, ß7, eß6) during the cycle will increase. At the beginning of the cycle no endometrial ehCG can be detected. In the middle and in the later phase as well as in the pre-decidual phase of the secretory cycle the ehCG can be detected.

The middle luteal phase of the cycle, as is well known, is the phase in which the small window of implantation is present in which the woman can become pregnant. The ovulation occurs about day 14. The fertilization can take place in the first 12 hours after ovulation. The window of implantation in which the embryo can implant in the endometrium is between day 20 and day 24 of the cycle.

When in the endometrium of the secretory phase of the cycle no hCG can be detected, a natural or artificial fertilization has no chance of success.

The detection of endometrial expression can be realized by isolation of RNA from different sources i.e., tissue or blood samples. Especially preferred is the detection in cells collected from the uterine cavity or cervical channel (see page 13, at the top) or menstrual blood (paragraph bridging pages 13-14).

An important application of the method according to the invention is the prediction of the chances of success of implantation of embryos created by in vitro fertilization. In case of in vitro fertilization, first hormonal stimulation by FSH (follicle stimulating hormone) is done for 10 to 14 days and then ovulation is triggered by administering hCG. The removal of the eggs by puncture is done approximately 36 hours later (shortly before rupture of the follicle). Now the actual in vitro fertilization takes place, i.e., addition of sperm or introduction of sperm into the ovum. The embryo transfer is carried out two to three days later when the embryo has a cell stage of 6 to 8 cells.

The detection of expression of hCG (ß6, ß7, eß6) is generally done after the removal of the egg by puncture, preferably shortly before the embryo been obtained by in vitro fertilization is to be implanted into the uterus. When no hCG (ß6, ß7, eß6) has been detected, the implantation chances are minimal. The embryos can be frozen and can be used in a subsequent receptive cycle. In order to do so, a hormone therapy can be performed.

Alternatively, the detection is done prospectively, i.e., by predicting the chances of success of implantation in a future cycle by detection of expression in the menstrual blood. In the menstrual blood cells of the endometrium as well as of the endocervix are present.

On page 13 and in the Examples 1 to 3 it is specifically explained how the RNA is isolated and how the detection of hCG (ß6, ß7, eß6) is done preferably by RT PCR or by other methods.

Based on the disclosure of the instant specification, a person skilled in the art is made aware that hCG (ß6/ß7/eß6) expression in the endometrium is correlated with the receptivity of the endometrium for implantation of an embryo. The disclosure provided in the instant specification enables a person skilled in the art to perform the diagnosis and to draw the proper conclusions: no hCG (ß6/ß7/eß6) - no receptivity; hCG (ß6/ß7/eß6) present - implantation possible / optimal.

The scientific findings underlying the present invention are based on the determination of expression of hCG in tissue containing decidua at early pregnancy stages at the mRNA and protein levels in samples taken from patients suffering from spontaneous abortion or ectopic pregnancies and the comparison to samples from women with normal pregnancies that were aborted for social/personal reasons. Inventors observed the absence of hCG mRNA levels in tissue samples from patients with spontaneous abortions or ectopic pregnancies when compared to pregnant women having a normal pregnancy. Patients suffering spontaneous abortions or ectopic pregnancies had non-detectable hCG expression (median intensity = 0) in the endometrium/decidua. In contrast, women with normal pregnancies showed a very high expression of hCG in decidua cells of decidua parietalis and basalis (median intensity of 17 patients = 2.5 for dec. parietalis and 4 for dec. basalis). The implantation of the fertilized egg occurs in the decidua basalis and therefore essential tolerance mechanisms allowing the acceptance of the fetus are expected to be in this area. Interestingly, the inventors observed a significant difference regarding hCG expression in decidua basalis from patients suffering from spontaneous abortion (median intensity = 0) or ectopic pregnancies (median intensity = 0) as compared to normal pregnancy (median intensity = 4).

Examiner states that no one in the art has studied whether any correlation exists between the expression of any of the ß7, ß6, eß6 hCG and endometrial receptivity. If such studies had been done and published, the instant invention would not be novel. Applicants have recognized such correlation and based on their scientific findings have presented a method for determining receptivity of the endometrium for implantation that is properly enabled by the instant specification.

Reconsideration and withdrawal of the rejection of the claims under 35 USC 112 are therefore respectfully submitted.

CONCLUSION

In view of the foregoing, it is submitted that this application is now in condition for allowance and such allowance is respectfully solicited.

Should the Examiner have any further objections or suggestions, the undersigned would appreciate a phone call or **e-mail** from the examiner to discuss appropriate amendments to place the application into condition for allowance.

Authorization is herewith given to charge any fees or any shortages in any fees required during prosecution of this application and not paid by other means to Patent and Trademark Office deposit account 50-1199.

Respectfully submitted on November 10, 2008,

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J Leukoc Biol. 2008 Apr;83(4):894-901. Epub 2008 Jan 2

Chorionic gonadotropin induces dendritic cells to express a tolerogenic phenotype.

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The pregnancy hormone human chorionic gonadotropin (hCG) has been suggested to play an immunoregulatory role in addition to its endocrine function, thus contributing to the prevention of fetal rejection. We hypothesized that hCG is involved in the maternal-fetal immune tolerance by the regulation of dendritic cell (DC) function. Therefore, we studied the effect of hCG on DC maturation. Upon hCG treatment in combination with LPS, mouse bone marrow-derived DC (BMDC) increased the ratio of IL-10:IL-12p70, down-regulated TNF-alpha, and decreased antigen-specific T cell proliferation. Addition of hCG together with LPS and IFN-gamma blocked MHC class II up-regulation, increased IL-10 production, and decreased the antigen-specific T cell proliferation by DC. Splenic DC showed similar results. Upon hCG treatment, IDO mRNA expression and its metabolite kynurenine were increased by LPS- and IFN-gamma-stimulated DC, suggesting its involvement in the decreased T cell proliferation. To study the effect of hCG on DC differentiation from precursors, BMDC were generated in the continuous presence of hCG. Under this condition, hCG decreased cytokine production and the induction of T cell proliferation. These data are suggestive for a contribution of hCG to the maternal-fetal tolerance during pregnancy by modifying DC toward a tolerogenic phenotype.